Gel/PCR DNA Fragments Extraction Kit

For research use only

Sample: up to 300 mg of agarose gel, up to 100 µl of PCR products

Fragment Size: 70 bp – 20 kb

Recovery: up to 95%

Format: spin column

Operation time: 20 minutes (gel extraction), 10 minutes (PCR cleanup)

Elution volume: 20-50 µl

CERTIFICATE NO. QAIC/TW/50077

ISO 9001:2008 QMS

Introduction

The Gel/PCR DNA Fragments Extraction Kit was designed to recover or concentrate DNA fragments (70 bp-20 kb) from agarose gel, PCR, or other enzymatic reactions in one convenient product. Chaotropic salt is used to dissolve agarose gel and denature enzymes. DNA fragments in chaotropic salt are bound by the glass fiber matrix of the spin column (1). Contaminants are removed with a Wash Buffer (containing ethanol) and the purified DNA fragments are eluted by a low salt Elution Buffer or TE. Salts, enzymes and unincorporated nucleotides can be effectively removed from the reaction mixture, without phenol extraction or alcohol precipitation. Typically, recoveries are up to 90% for Gel Extraction and up to 95% for PCR Clean up. The eluted DNA is ready for use in PCR, Fluorescent or Radioactive Sequencing, Restriction Enzyme Digestion, DNA Labeling and Ligation.

Quality Control

The quality of the Gel/PCR DNA Fragments Extraction Kit is tested on a lot-to-lot basis by isolating DNA fragments of various sizes from either aqueous solutions or agarose gel. The purified DNA is analyzed by electrophoresis.

Kit Contents

Name	DF004	DF100	DF300
DF Buffer	3 ml	80 ml	240 ml
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer* (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml + 25 ml (200 ml) (100 ml)
Elution Buffer (10 mM Tris-HCl, pH 8.5 at 25°C)	1 ml	6 ml	30 ml
DF Column	4 pcs	100 pcs	300 pcs
2 ml Collection Tube	4 pcs	100 pcs	300 pcs

IMPORTANT BEFORE USE!

*Add absolute ethanol (see the bottle label for details) to the Wash Buffer prior to initial use

Caution

DF Buffer contains guanidine thiocyanate. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615

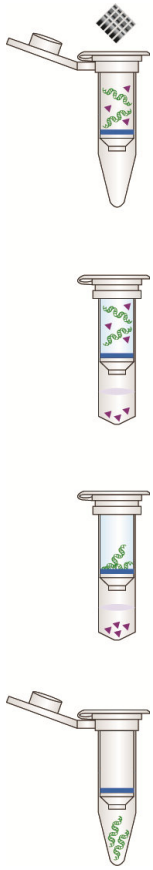
Additional requirements

microcentrifuge tubes, absolute ethanol

Gel Extraction Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use

Gel Dissociation	• Excise the agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice (Use only TAE buffer for gel formation). • Transfer up to 300 mg of the gel slice to a 1.5 ml microcentrifuge tube. • Add **500 µl of DF Buffer** to the sample and mix by vortex. • Incubate at 55-60ºC for 10-15 minutes to ensure the gel slice has been completely dissolved. During incubation, invert the tube every 2-3 minutes. • Cool the dissolved sample mixture to room temperature.
Step 1 DNA Binding	• Place the **DF Column** in a **2 ml Collection Tube**. • Transfer 800 µl of the sample mixture to the **DF Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the **DF Column** back in the **2 ml Collection Tube**. NOTE: If the sample mixture is more than 800 µl, repeat the DNA Binding step.
Step 2 Wash	• Add **400 µl of W1 Buffer** into the **DF Column**. • Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. • Place the **DF Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (make sure ethanol was added) into the **DF Column**. • Let stand for 1 minute at room temperature. • Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. • Place the **DF Column** back in the **2 ml Collection Tube**. • Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.
Step 3 DNA Elution	• Transfer the dried **DF Column** to a new 1.5 ml microcentrifuge tube. • Add **20-50 µl of Elution Buffer** or TE into the center of the column matrix. • Let stand for at least 2 minutes to ensure the **Elution Buffer** is completely absorbed. • Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.



Gel Extraction (Sequencing) Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use

Gel Dissociation	• Excise the agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice (Use only TAE buffer for gel formation). • Transfer up to 300 mg of the gel slice to a 1.5 ml microcentrifuge tube. • Add **500 µl of DF Buffer** to the sample and mix by vortex. • Incubate at 55-60ºC for 10-15 minutes to ensure the gel slice has been completely dissolved. During incubation, invert the tube every 2-3 minutes. • Cool the dissolved sample mixture to room temperature.
Step 1 DNA Binding	• Place the **DF Column** in a **2 ml Collection Tube**. • Transfer 800 µl of the sample mixture to the **DF Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the **DF Column** back in the **2 ml Collection Tube**. NOTE: If the sample mixture is more than 800 µl, repeat the DNA Binding Step.
Step 2 Wash	• Add **600 µl of Wash Buffer** (make sure ethanol was added) into the **DF Column** and let stand for 1 minute. • Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. • Place the **DF Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (make sure ethanol was added) into the **DF Column** and let stand for 1 minute. • Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. • Place the **DF Column** back in the **2 ml Collection Tube**. • Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.
Step 3 DNA Elution	• Transfer the dried **DF Column** to a new 1.5 ml microcentrifuge tube. • Add **20-50 µl of Elution Buffer** or TE into the center of the column matrix. • Let stand for at least 2 minutes to ensure the **Elution Buffer** is completely absorbed. • Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.

PCR Clean Up Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use

Sample Prep.	• Transfer up to 100 µl of reaction product to a 1.5 microcentrifuge tube. • Add **5 volumes of DF Buffer** to 1 volume of the sample and mix by vortex.
Step 1 DNA Binding	• Place a **DF Column** in a **2 ml Collection Tube**. • Transfer the sample mixture to the **DF Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the **DF Column** back in the **2 ml Collection Tube**.
Step 2 Wash	• Add **600 µl of Wash Buffer** (make sure ethanol was added) into the center of the **DF Column**. • Let stand for 1 minute at room temperature. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the **DF Column** back in the **2 ml Collection Tube**. • Centrifuge for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 3 DNA Elution	• Transfer the dried **DF Column** to a new 1.5 ml microcentrifuge tube. • Add **20-50 µl of Elution Buffer** or TE into the center of the column matrix. • Let stand for at least 2 minutes to ensure the **Elution Buffer** is completely absorbed. • Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	**Gel slice did not dissolve completely** • The Gel slice was too big. If using more than 300 mg of gel slice, separate it into multiple tubes. • Raise the incubation temperature to 60ºC and extend the incubation time.
	Incorrect DNA Elution Step • Ensure that the Elution Buffer is completely absorbed after being added to the center of the DF Column.
	Incomplete DNA Elution • If the DNA fragments are larger than 10 kb, use pre-heated Elution Buffer (60-70ºC) in the Elution Step to improve the elution efficiency.
Eluted DNA doesn't perform well in downstream applications	**Residual ethanol contamination** • Following the Wash Step, dry the DF Column with additional centrifugation at 14-16,000 x g for 5 minutes or incubate at 60ºC for 5 minutes.
	DNA was denatured (a smaller band appeared on gel analysis) • Incubate the eluted DNA at 95ºC for 2 minutes, and then cool down slowly to re-anneal the denatured DNA.
Low A260/A230	• In the wash step, repeat the 600 µl of Wash Buffer addition and let stand for 1 minute.

Gel/PCR DNA Fragments Extraction Kit Functional Test Data

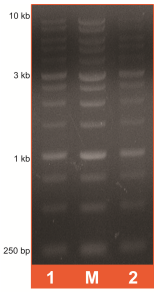


Figure 1. Gel slice DNA fragments ranging from 250 bp-10 kb were extracted using both the Gel/PCR DNA Fragments Extraction Kit (lane 1) and the equivalent competitor's gel extraction kit (lane 2). The purified DNA from a 50 µl eluate was analyzed by electrophoresis on a 1% agarose gel.

M = Geneaid 1 Kb DNA Ladder

Test	Yield	260/280	260/230	Recovery
Geneaid	6.40 µg/ml	1.83	1.67	92%
	6.50 µg/ml	1.84	1.71	94%
Competitor	6.30 µg/ml	1.85	0.45	91%
	6.50 µg/ml	1.83	0.51	94%

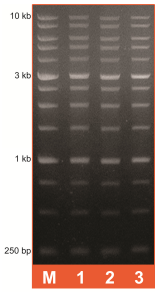


Figure 2. PCR product DNA fragments ranging from 250 bp-10 kb were extracted using the Gel/PCR DNA Fragments Extraction Kit (total 1.1 µg, 3 replicates). The purified DNA concentration from a 50 µl eluate was confirmed by spectrophotometer and analyzed by electrophoresis on a 1% agarose gel.

M = Geneaid 1 Kb DNA Ladder

Test		DNA	Total DNA	Recovery
M	Control	110 ng/ml	1100 ng	-----
1	Test 1	20.8 9g/ml	1040 ng	95%
2	Test 2	20.3 ng/ml	1015 ng	92%
3	Test 2	19.6 ng/ml	980 ng	89%

Related DNA/RNA Purification Products

Plasmid DNA Purification			
Product	Package Size	Cat. Number	Specifications
Presto™ Mini Plasmid Kit	100/300 preps	PDH100/300	1-7 ml of cultured bacterial cells
Presto™ Midi Plasmid Kit	25 preps	PIF025	50-150 ml of cultured bacterial cells
High-Speed Plasmid Mini Kit (10-50 Kb)	25 preps	PDL100/300	optimized for 10-50 kb plasmid
High-Speed Plasmid Advance Kit (50-100 ml)	25 preps	PA025	large volume spin column
Geneaid™ Maxi Plasmid Kit	10/25 preps	PM010/25	200-400 ml of cultured bacterial cells
Presto™ 96 Well Plasmid Kit	4/10 x 96 preps	96PDV04/10	high-throughput plasmid DNA purification
Gel Extraction and PCR Cleanup			
Product	Package Size	Cat. Number	Specifications
Gel/PCR DNA Fragments Extraction Kit	100/300 preps	DF100/300	70 bp – 20 kb DNA fragments
Gel/PCR DNA Fragments Extraction Maxi Kit	10/25 preps	DM010/025	up to 6 g of gel or 2 ml of PCR product
Small DNA Fragments Extraction Kit	100/300 preps	DF101/301	40 bp – 200 bp DNA fragments
Presto™ Max Gel/PCR Kit (Large DNA Fragments)	100/300 preps	DFL100/300	100 bp – 30 kb DNA fragments
Presto™ 96 Well PCR Cleanup Kit	4/10 x 96 preps	96DFH04/10	High-throughput PCR cleanup
Genomic DNA Purification			
Product	Package Size	Cat. Number	Specifications
Geneius™ Micro gDNA Extraction Kit	100 preps	GMB100	small sample volumes
Presto™ Buccal Swab gDNA Extraction Kit	100 preps	GSK100	up to 2 µg of pure gDNA per buccal swab
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300	RBC Lysis Buffer DNA purification
Genomic DNA Midi Kit (Blood/Cultured Cell)	25 preps	GDI25	up to 2 ml of whole blood, cultured cells (up to 5 x 10^7)
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM10/25	up to 10 ml of frozen blood, cultured cells (up to 1 x 10^8)
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300	Provided micropestle tissue homogenization
Genomic DNA Mini Kit (Plant)	100 preps	GP100	up to 100 mg (fresh plant), 25 mg (dry plant)
Presto™ Mini gDNA Yeast Kit	100/300 preps	GBY100/300	includes Sorbitol Buffer
Presto™ Mini gDNA Bacteria Kit	100/300 preps	GBB100/300	includes Gram+ Buffer
gSYNC™ DNA Extraction Kit	50/100/300 preps	GS050/100/300	Proteinase K digestion
Total RNA Purification			
Product	Package Size	Cat. Number	Specifications
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	RB050/100/300	up to 300 µl of whole blood, cultured cells (up to 5 x 10^6)
Total RNA Mini Kit (Tissue)	50/100/300 preps	RT050/100/300	provided micropestle tissue homogenization
Total RNA Mini Kit (Plant)	50/100/300 preps	RP050/100/300	up to 100 mg (fresh plant), up to 25 mg (dry plant)
Presto™ Mini RNA Bacteria Kit	50/100/300 preps	RBB050/100/300	includes Gram+ Buffer
Presto™ Mini RNA Yeast Kit	50/100/300 preps	RBY050/100/300	includes Sorbitol Buffer
miRNA Isolation Kit	50/100 preps	RMI050/100	high yield miRNA and other small RNA
GENEzol™ Reagent	50/100/200 ml	GZR050/100/200	simultaneous extraction of RNA, DNA and protein
GENEzol™ TriRNA Pure Kit	50/100/200 preps	GZX050/100/200	phenol and guanidine isothiocyanate plus spin column system for convenient purification of high-quality total RNA
TriRNA Pure Kit	50/100/200 preps	TRP050/100/200	spin column system for convenient purification of high-quality total RNA to be used with GENEzol™ or TRIzol®